

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
17 June 2004 (17.06.2004)

PCT

(10) International Publication Number  
WO 2004/050910 A1

(51) International Patent Classification<sup>7</sup>: C12Q 1/68,  
G01N 33/543

(21) International Application Number:  
PCT/EP2003/013601

(22) International Filing Date: 2 December 2003 (02.12.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
02447241.7 4 December 2002 (04.12.2002) EP  
60/440,689 17 January 2003 (17.01.2003) US

(71) Applicant (for all designated States except US): PAM-  
GENE BV [NL/NL]; Burgemeester Loeffplein 70a,  
NL-5211 RX Den Bosch (NL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): WU, Ying [NL/NL];  
Lankforst 14-76, NL-6538 JC Nijmegen (NL). VAN  
BEUNINGEN, Marinus, Gerardus, Johannes [NL/NL];  
Polderkade 27, NL-5345 RR Oss (NL). CHAN, Alan  
[GB/GB]; 4 Northolt Avenue, Ruislip, Middlesex HA4  
6ST (GB).

(74) Agents: DE CLERCQ, Ann et al.; De Clercq, Brants &  
Partners CV, E. Gevaertdreef 10a, B-9830 Sint-Martens-  
Latem (BE).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR,  
CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,  
GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR,  
KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN,  
MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU,  
SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA,  
UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (BW, GH,  
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),  
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,  
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE,  
SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA,  
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Declaration under Rule 4.17:**

— of inventorship (Rule 4.17(iv)) for US only

**Published:**

— with international search report  
— before the expiration of the time limit for amending the  
claims and to be republished in the event of receipt of  
amendments

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

WO 2004/050910 A1

(54) Title: METHOD FOR HYBRIDISATION OF IMMOBILIZED GENOMIC DNA

(57) Abstract: The present invention is directed to a novel method of efficiently hybridising probes onto immobilized genomic DNA and/or RNA comprising the steps of (a) providing intact genomic DNA and denaturing said intact genomic DNA; (b) immobilizing said denatured intact genomic DNA onto a matrix; said matrix comprising pore sizes within a range of 0.6 µm to 2 µm including the outer limits (c) providing a set of probes and passing said probes through said matrix under conditions favouring hybridisation of the probes to its complementary sequence in said intact genomic DNA; and (d) washing off non-hybridised probes through said matrix, leaving formed hybridised intact genomic DNA/probe complexes for further analysis. The present invention is further directed to a novel method for target nucleic acid detection and quantification in a genomic DNA sample comprising the steps of: (a) providing intact genomic DNA and denaturing said intact genomic DNA; (b) performing a hybridisation according to a method as described above; (c) recovering hybridised probes; and essentially simultaneously amplifying any recovered probe using a single primer pair, each member of said primer pair binding to each recovered probe onto the respective flanking primer attachment sequences of said probe, and (d) qualitatively and quantitatively analysing the recovered amplified probes of step (c). The present invention also relates to the uses thereof as well as devices, apparatus and kits for performing said methods of the invention.

BEST AVAILABLE COPY